Supporting information for

How Does the Reductase Help Regulating the Catalytic Cycle of Cytochrome P450 3A4 Using the Conserved Water Channel?

Dan Fishelovitch, Sason Shaik, Haim J. Wolfson, Ruth Nussinov

Table of contents:

1. Ferric heme topology

2. Testosterone topology file

3. Ferric heme QM/MM optimized coordinates

4. Additional figures

5. Calculated *PKa* values

1. Ferric heme topology

```
RESI HEC2     -2.00
!cysteine
GROUP
ATOM N    NH1   -0.57!     |
ATOM HN   H      0.40!  HN-N
ATOM CA   CT1   -0.01!     |   HB1
ATOM HAY  HB     0.18!     |   |
GROUP               ! HAY-CA--CB--SG   move definition of HA-->HAY
ATOM CB   CS    -0.37!     |   |     \
ATOM HB1  HA     0.20!     |   HB2    (FE)
ATOM HB2  HA     0.20!   O=C
ATOM SG   SS    -0.03!     |
GROUP
ATOM C    C      0.50
ATOM O    O     -0.50
GROUP
ATOM FE   FE     0.74!       O2A   O1A             O2D  O1D
ATOM NA   NPH   -0.77!         \\ //                 \\ //
ATOM NB   NPH   -0.77!          CGA                   CGD
ATOM NC   NPH   -0.77!           |                     |
ATOM ND   NPH   -0.77!    HBA1--CBA--HBA2  HA   HBD1--CBD--HBD2
ATOM C1A  CPA    0.27!           |          |          |
ATOM C2A  CPB    0.04!    HAA1--CAA-HAA2  _CHA_ HAD1--CAD--HAD2
ATOM C3A  CPB    0.04!           |       /     \       |
ATOM C4A  CPA    0.27!          C2A---C1A       C4D---C3D
ATOM C1B  CPA    0.27!           |     |         |     |
ATOM C2B  CPB    0.04!HMA1\      |     |         |     |      /HMD1
ATOM C3B  CPB   -0.04!HMA2-CMA--C3A    NA       ND    C2D--CMD-HMD2
ATOM C4B  CPA    0.27!HMA3/       \   / \       / \   /       \HMD3
ATOM C1C  CPA    0.27!             C4A   \     /   C1D
ATOM C2C  CPB    0.04!            /       \   /       \
ATOM C3C  CPB   -0.02!      HB--CHB        FE         CHD--HD
ATOM C4C  CPA    0.27!           \       /   \       /
ATOM C1D  CPA    0.27!            C1B   /     \   C4C        HAC
ATOM C2D  CPB    0.04!HMB1\      /   \ /       \ /   \       /
ATOM C3D  CPB    0.04!HMB2-CMB--C2B    NB       NC    C3C--CAC
ATOM C4D  CPA    0.27!HMB3/      |     |         |     |     \\  /HBC1
GROUP               !           |     |         |     |      CBC
ATOM CHA  CPM   -0.13!          C3B---C4B       C1C---C2C        \HBC2
ATOM HA   HA     0.13!           |       \_CHC_/       |
GROUP               !          CAB         |         CMC--HMC3
ATOM CHB  CPM   -0.13!         //  \        HC       /  |
ATOM HB   HA     0.13!        CBB  HAB           HMC1  HMC2
GROUP               !       /   \
ATOM CHC  CPM   -0.13!    HBB1  HBB2
ATOM HC   HA     0.13!
GROUP
ATOM CHD  CPM   -0.13
ATOM HD   HA     0.13
GROUP
ATOM CMA  CT3   -0.48
ATOM HMA1 HA     0.16
ATOM HMA2 HA     0.16
ATOM HMA3 HA     0.16
GROUP
ATOM CAA  CT2   -0.30
ATOM HAA1 HA     0.15
```

```
ATOM HAA2 HA     0.15
GROUP
ATOM CBA  CT2   -0.34
ATOM HBA1 HA     0.15
ATOM HBA2 HA     0.15
ATOM CGA  CC     0.50
ATOM O1A  OC    -0.73
ATOM O2A  OC    -0.73
GROUP
ATOM CMB  CT3   -0.48
ATOM HMB1 HA     0.16
ATOM HMB2 HA     0.16
ATOM HMB3 HA     0.16
GROUP
ATOM CAB  C     -0.20
ATOM HAB  HA     0.20
GROUP
ATOM CBB  C     -0.30
ATOM HBB1 HA     0.15
ATOM HBB2 HA     0.15
GROUP
ATOM CMC  CT3   -0.48
ATOM HMC1 HA     0.16
ATOM HMC2 HA     0.16
ATOM HMC3 HA     0.16
GROUP
ATOM CAC  C     -0.20
ATOM HAC  HA     0.20
GROUP
ATOM CBC  C     -0.30
ATOM HBC1 HA     0.15
ATOM HBC2 HA     0.15
GROUP
ATOM CMD  CT3   -0.48
ATOM HMD1 HA     0.16
ATOM HMD2 HA     0.16
ATOM HMD3 HA     0.16
GROUP
ATOM CAD  CT2   -0.30
ATOM HAD1 HA     0.15
ATOM HAD2 HA     0.15
GROUP
ATOM CBD  CT2   -0.34
ATOM HBD1 HA     0.15
ATOM HBD2 HA     0.15
ATOM CGD  CC     0.50
ATOM O1D  OC    -0.73
ATOM O2D  OC    -0.73
```

Fe and S related missing parameters were added from Autenrieth et al. (Autenrieth F, Tajkhorshid E, Baudry J, Luthey-Schulten Z (2004) Classical force field parameters for the heme prosthetic group of cytochrome c. *J Comput Chem* 25:1613-1622) and Bathelt et al. (Bathelt CM, Zurek J, Mulholland AJ, Harvey JN (2005) Electronic structure of compound I in human isoforms of cytochrome P450 from QM/MM modeling. *J Am Chem Soc* 127:12900-12908).

2. Testosterone topology file

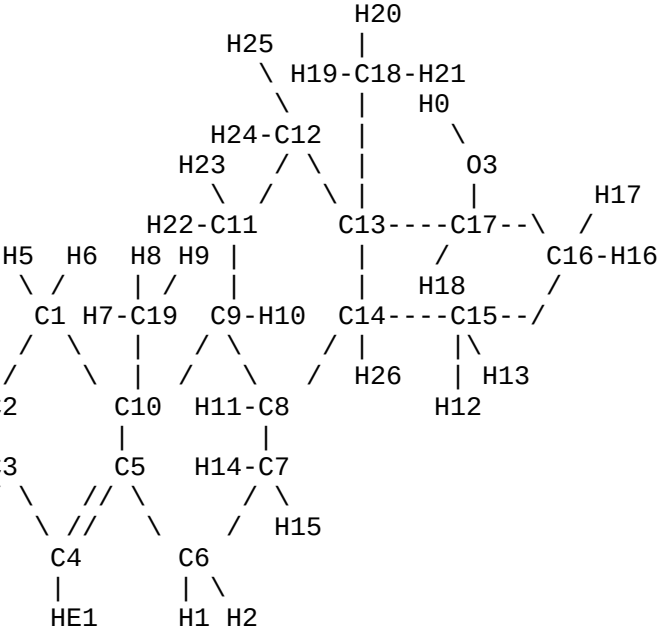
```
*  testosterone 6oh topology
*
MASS   93  HAL1  1.008000 H ! alphatic proton
MASS   94  HAL2  1.008000 H ! alphatic proton
MASS   95  HAL3  1.008000 H ! alphatic proton
MASS   96  HEL1  1.00800 H  ! for alkene; RHC=CR
MASS   62  HOL   1.00800 H  ! for H in hydroxyl group
MASS   65  CTL1 12.011000 C ! sp3 carbon with 1 H  (-CH1-)
MASS   66  CTL2 12.011000 C ! carbon of methylene group (-CH2-)
MASS   67  CTL3 12.011000 C ! carbon of methyl group (-CH3)
MASS   97  CEL1 12.011000 C ! for alkene; RHC=CR
MASS   15  CEL2 12.011000 C ! for alkene; H2C=CR
MASS  69   OHL   15.99940 O ! hydroxyl oxygen

DECL -CA
DECL -C
DECL -O
DECL +CA

DEFAULT FIRST NONE LAST NONE

AUTO ANGLES DIHE

RESI TSTR  0.000000
GROUP
ATOM  C3   C   0.51
ATOM  O2   O  -0.51
GROUP
ATOM  C5   CEL1 -0.092
ATOM  HE1  HEL1  0.088
ATOM  C4   CEL1 -0.083
ATOM  C10  CTL1  0.087
GROUP
ATOM  C6   CTL1 -0.108
ATOM  H2   HAL1  0.054
ATOM  H1   HAL1  0.054
GROUP
ATOM  C14  CTL1 -0.204
ATOM  H26  HAL1  0.121
ATOM  C13  CTL1  0.083
GROUP
ATOM  H10  HAL1  0.104
ATOM  C9   CTL1 -0.159
ATOM  C11  CTL2  0.036
ATOM  H22  HAL2  0.012
ATOM  H23  HAL2  0.007
GROUP
ATOM  H14  HAL2  0.103
ATOM  H15  HAL2  0.091
ATOM  C7   CTL2 -0.187
ATOM  C8   CTL1 -0.190
ATOM  H11  HAL1  0.183
GROUP
ATOM  C17  CTL1 -0.008
ATOM  H18  HAL1  0.180
ATOM  O3   OHL  -0.566
```



```
ATOM  HO   HOL   0.394
GROUP
ATOM  C16  CTL2 -0.108
ATOM  H17  HAL2  0.054
ATOM  H16  HAL2  0.054
GROUP
ATOM  C18  CTL3 -0.018
ATOM  H19  HAL3  0.006
ATOM  H20  HAL3  0.006
ATOM  H21  HAL3  0.006
GROUP
ATOM  C2   CTL2 -0.162
ATOM  H3   HAL2  0.081
ATOM  H4   HAL2  0.081
GROUP
ATOM  C1   CTL2 -0.118
ATOM  H5   HAL2  0.059
ATOM  H6   HAL2  0.059
GROUP
ATOM  C19  CTL3 -0.144
ATOM  H7   HAL3  0.048
ATOM  H8   HAL3  0.048
ATOM  H9   HAL3  0.048
GROUP
ATOM  C12  CTL2  0.036
ATOM  H24  HAL2 -0.018
ATOM  H25  HAL2 -0.018
GROUP
ATOM  C15  CTL2 -0.134
ATOM  H12  HAL2  0.067
ATOM  H13  HAL2  0.067
BOND C1  H5    C1  H6    C1  C2   C1  C10
BOND C2  H4    C2  H3    C2  C3
BOND C3  O2    C3  C4
BOND C4  HE1   C4  C5
BOND C5  C10   C5  C6
BOND C6  H1    C6  C7    C6  H2
BOND C7  H15   C7  H14   C7  C8
BOND C8  H11   C8  C9    C8  C14
BOND C9  H10   C9  C10   C9  C11
BOND C10 C19
BOND C11 H22   C11 H23   C11 C12
BOND C12 H24   C12 H25   C12 C13
BOND C13 C18   C13 C17   C13 C14
BOND C14 C15   C14 H26
BOND C15 H12   C15 H13   C15 C16
BOND C16 C17   C16 H17   C16 H16
BOND C17 H18   C17 O3
BOND C18 H19   C18 H20   C18 H21
BOND C19 H7    C19 H8    C19 H9    O3  HO
IMPR C19 C9 C10 C5
IMPR C18 C17 C13 C14
IMPR C12 C17 C13 C18
IMPR C17 C14 C13 C18
IMPR C14 C12 C13 C18
IMPR O3 C16  C17  C13
IMPR C4  C10 C5  C6
IMPR H11 C9 C8 C14
IMPR C15 C13 C14 C8
IMPR C4  C5  C3  HE1
IMPR C6  C4  C5  C10
```

```
IMPR C2  C4   C3  O2

PATCHING FIRS NONE LAST NONE

IC O2   C3   C4   C5    1.2344  121.6394 -179.2938  123.5448  1.3539
IC O2   C3   C4   HE1   1.2344  121.6394   -6.8737  115.8687  1.0586
IC C2   C3   C4   C5    1.5028  117.4983   -1.4695  123.5448  1.3539
IC O2   C3   C2   C1    1.2344  120.8259 -150.9922  111.3406  1.5138
IC O2   C3   C2   H3    1.2344  120.8259  -20.4986  115.4453  1.0014
IC O2   C3   C2   H4    1.2344  120.8259   94.4287  101.3031  0.8804
IC C2   C4  *C3   O2    1.5010  117.40    177.80    121.70    1.2320
IC C3  C5  *C4   HE1   1.5248  124.79    179.94    118.57    1.0971
IC C4   C3   C2   H3    1.4481  117.4983  161.6584  115.4453  1.0014
IC C3   C4   C5   C10   1.4481  123.5448   -7.0199  121.9877  1.5327
IC C3   C4   C5   C6    1.4481  123.5448  170.4017  120.3593  1.4904
IC C4   C5   C10  C9    1.3539  121.9877 -132.3047  107.0264  1.5651
IC C4   C5   C10  C19   1.3539  121.9877  106.2116  108.0069  1.5413
IC C4   C5   C6   H1    1.3539  120.3593    6.8895  105.7211  0.9895
IC C4   C5   C6   H2    1.3539  120.3593 -112.6151  107.0076  0.9644
IC C4   C5   C6   C7    1.3539  120.3593  130.4844  111.7356  1.5434
IC C10  C4  *C5   C6    1.5532  116.94   -179.98    120.08    1.3679
IC C5   C10  C9   C11   1.5327  107.0264  178.1346  112.1120  1.5548
IC C5   C10  C9   H10   1.5327  107.0264   60.8136  108.3432  1.0828
IC C5   C10  C9   C8    1.5327  107.0264  -52.7866  113.4307  1.5440
IC C5   C10  C1   C2    1.5327  110.3179   44.8338  114.1190  1.5138
IC C5   C10  C1   H5    1.5327  110.3179  -74.5786  104.4856  1.0064
IC C5   C10  C1   H6    1.5327  110.3179  170.4465  105.5003  0.9185
IC C7  H2  *C6   C5    1.5554  109.79    121.62    109.95    1.4930
IC C5  H2  *C6   H1    1.5484  109.95    115.61    103.27    1.4814
IC H2  C6   C7   C8    0.9670  109.79    -63.5     111.81    1.5384
IC C1   C10  C19  H9    1.5394  110.5418  -61.5091  106.6646  0.9094
IC C5   C10  C19  H7    1.5327  108.0069   60.8179  110.7569  1.0385
IC C5   C10  C19  H8    1.5327  108.0069  -63.1393  109.5755  1.0585
IC C8  C10 *C9   C11   1.5715  112.46   -127.75    113.03    1.5661
IC C8  C10 *C9   H10   1.5715  112.46    115.20    106.10    1.1100
IC C9  C5  *C10  C19   1.5860  110.19   -122.48    108.65    1.5717
IC C9  C5  *C10  C1    1.5860  110.19    118.58    108.33    1.5792
IC C5   C6   C7   C8    1.4904  111.7356   53.1650  110.7696  1.5307
IC C5   C6   C7   H14   1.4904  111.7356  -69.4176  112.7754  0.9473
IC C5   C6   C7   H15   1.4904  111.7356  168.4643  109.9922  1.0403
IC C6  C8  *C7   H15   1.5248  112.71   -122.34    110.04    1.1056
IC C6  C8  *C7   H14   1.5248  112.71    121.93    109.40    1.1103
IC C10  C9   C8   C14   1.5860  112.46   -177.70    109.53    1.5530
IC C14  C9  *C8   C7    1.5530  109.53   -121.29    109.65    1.5572
IC C7  C9  *C8   H11   1.5572  109.65   -118.43    109.45    1.1058
IC C9  C8   C14  C13   1.5715  109.53     57.19    115.16    1.5733
IC C13  C8  *C14  C15   1.5733  115.16    124.15    118.45    1.5558
IC C8   C14  C13  C17   1.5198  113.3538  177.8566   99.1606  1.5528
IC C13  C8  *C14  H26   1.5733  115.16   -116.19    106.00    1.1112
IC H26  C14  C13  C17   1.1456  102.1091  -65.8025   99.1606  1.5528
IC H26  C14  C13  C18   1.1456  102.1091  179.1162  113.1591  1.5232
IC H26  C14  C13  C12   1.1456  102.1091   54.2176  108.0403  1.5308
IC C15  C14  C13  C17   1.5392  104.2003   46.7414   99.1606  1.5528
IC C17  C14 *C13  C18   1.5861  100.15   -117.12    112.56    1.5646
IC C18  C14 *C13  C12   1.5646  112.56   -121.33    106.26    1.5620
IC C8  C14  C13  C17   1.5530  115.16    178.17    100.15    1.5861
IC C13  C14  C8   C9    1.5431  113.3538   58.2605  109.1035  1.5440
IC H26  C14  C8   H12   1.1456  108.3693 -173.4467  107.8725  0.9743
IC C13  C14  C15  C16   1.5431  104.2003  -32.9379  104.1198  1.5330
IC H26  C14  C15  H12   1.1456  108.1766 -161.7415  110.2486  1.0435
IC H26  C14  C15  H13   1.1456  108.1766  -29.6121  106.7957  1.0170
```

```
IC C16  C14 *C15  H12   1.5750  103.65    119.22    110.78    1.1042
IC H12  C14 *C15  H13   1.1042  110.78    119.69    112.42    1.1025
IC C14  C13  C17  O3    1.5431  99.1606  -161.5404  114.9010  1.4228
IC C14  C13  C17  H18   1.5431  99.1606    73.5699  113.4379  1.0862
IC C16  C13 *C17  H18   1.5879  103.30    113.79    106.39    1.1096
IC C16  H18 *C17  C13   1.5554  109.79    121.62    113.95    1.5530
IC C13  H18 *C17  O3    1.5484  113.95    128.61    107.27    1.4214
IC C12  C13  C18  H21   1.5308  112.2326 -176.8153  110.6633  1.0498
IC C14  C13  C18  H19   1.5431  113.1591  -63.6430  110.5362  0.8845
IC C14  C13  C18  H20   1.5431  113.1591  171.4897  114.1382  0.9941
IC H19  C13 *C18  H20   1.1033  110.55   -119.68    111.78    1.1028
IC H19  C13 *C18  H21   1.1033  110.55    119.61    112.19    1.1018
IC H7   C10 *C19  H8    1.1021  111.45    119.42    110.93    1.1019
IC H7   C10 *C19  H9    1.1021  111.45   -119.79    111.35    1.1031
IC C14  C13  C12  C11   1.5431  108.0403   57.3169  110.8625  1.5401
IC C14  C13  C12  H24   1.5431  108.0403  -64.1758  105.2277  1.0268
IC C14  C13  C12  H25   1.5431  108.0403 -178.7801  111.4882  1.0753
IC C11  C13 *C12  H24   1.5634  111.58   -120.79    108.67    1.1085
IC C11  C13 *C12  H25   1.5634  111.58    121.62    111.81    1.1024
IC C10  C9   C11  C12   1.5651  112.1120  179.1474  113.1929  1.5401
IC C10  C9   C11  H22   1.5651  112.1120   58.2458  107.5491  1.0142
IC C10  C9   C11  H23   1.5651  112.1120  -62.4416  108.2043  1.1186
IC C12  C9  *C11   H22  1.5634  114.01   -121.93    109.80    1.1027
IC H22  C9  *C11   H23  1.1027  109.80   -116.32    109.56    1.1045
IC C10  C9   C8   C14   1.5651  113.4307 -179.3439  109.1035  1.5198
IC C13  C12  C11  C9    1.5308  110.8625  -53.1491  113.1929  1.5548
IC C6   C7   C8   C14   1.5434  110.7696 -177.4380  111.0609  1.5198
IC C16  C17  O3   HO    1.5589  109.4807  156.5484  110.1648  0.7841
IC H18  C17  O3   HO    1.1017  103.27    179.88    109.63    0.9872
IC C13  C17  C16  C15   1.5528  103.4469   23.2683  106.5419  1.5330
IC C13  C17  C16  H17   1.5528  103.4469  -97.5247  110.2519  1.0376
IC C13  C17  C16  H16   1.5528  103.4469  141.5424   99.9022  0.9963
IC C15  C17 *C16  H17   1.5750  107.12   -120.82    110.34    1.1040
IC C15  C17 *C16  H16   1.5750  107.12    121.24    110.92    1.1025
IC C14  C15  C16  C17   1.5392  104.1198    5.6394  106.5419  1.5589
IC C3   C2   C1   C10   1.5028  111.3406  -53.5258  114.1190  1.5394
IC C1  C3  *C2   H4    1.5582  110.59    121.34    109.37    1.1037
IC C1  C3  *C2   H3    1.5582  110.59   -121.75    109.32    1.1074
IC C1 H4  *C2   C3    1.5130  107.90   -117.11    101.30    1.5040
IC C3 H4  *C2   H3    1.5040  101.30   -121.00    107.00    1.0020
IC C2  C10 *C1   H5    1.5582  114.55    122.79    109.37    1.1042
IC H5 C10 *C1   H6    1.1042  109.37    116.06    108.25    1.1063
END
```

3. Ferric heme QM/MM optimized coordinates

```
C    -6.112457 -3.493490 -2.649526
O    -5.951633 -3.366412 -3.893706
N    -5.099632 -3.397687 -1.772787
H    -5.279463 -3.454247 -0.766625
C    -3.712096 -3.183504 -2.218481
H    -3.639012 -2.204990 -2.697933
C    -2.788894 -3.252839 -1.000446
H    -2.796202 -4.267367 -0.595739
H    -3.112452 -2.544623 -0.236693
C    -3.274285 -4.288512 -3.196361
O    -3.177906 -5.465934 -2.804069
S    -0.998201 -2.870267 -1.482340
FE   -1.043614 -0.464155 -1.562068
N    -2.826292 0.099093 -0.603055
N    -0.095528 -0.148154 0.289418
N    0.784053 -0.212449 -2.480374
N    -1.980856 0.006904 -3.373392
C    -4.064015 0.317649 -1.212722
C    -5.089728 0.461571 -0.194581
C    -4.462046 0.354849 1.028939
C    -3.055220 0.116469 0.773374
C    -0.709523 -0.200725 1.539322
C    0.284963 -0.400252 2.579693
C    1.518532 -0.469940 1.947467
C    1.263522 -0.315858 0.513756
C    2.019098 -0.362468 -1.859562
C    3.061998 -0.471763 -2.860851
C    2.450096 -0.344928 -4.099862
C    1.015057 -0.198793 -3.850946
C    -1.352581 0.065315 -4.608154
C    -2.337209 0.368824 -5.639431
C    -3.561695 0.508344 -5.012567
C    -3.321773 0.297410 -3.590528
C    -4.280163 0.403542 -2.585164
H    -5.296692 0.602232 -2.904307
C    -2.080197 -0.068396 1.750286
H    -2.423970 -0.093507 2.778533
C    2.230060 -0.405279 -0.482455
H    3.253691 -0.548082 -0.158103
C    0.018617 -0.093780 -4.819467
H    0.351895 -0.105322 -5.848632
C    -5.076111 0.523342 2.380214
H    -4.829051 -0.299083 3.058293
H    -6.166422 0.586638 2.317695
H    -4.718109 1.435231 2.871950
C    -6.554223 0.675660 -0.450132
H    -7.141177 0.177156 0.330813
H    -6.860674 0.193555 -1.383862
C    -6.985452 2.155689 -0.511722
H    -6.292039 2.725397 -1.147960
H    -6.941346 2.626007 0.479866
C    -8.384823 2.348176 -1.082793
O    -9.012927 1.338730 -1.558077
O    -8.856203 3.553046 -1.154562
C    -0.001334 -0.503311 4.046255
H    -0.026609 -1.548185 4.382718
H    -0.969240 -0.066433 4.308101
```

```
H    0.773152 0.009707 4.628297
C    2.847080 -0.654678 2.517692
H    3.677590 -0.304932 1.909799
C    3.145377 -1.216555 3.704641
H    2.400476 -1.629790 4.374618
H    4.176737 -1.299353 4.030180
C    4.517145 -0.678668 -2.562797
H    4.688266 -1.642860 -2.067174
H    4.913165 0.094559 -1.891786
H    5.128564 -0.659841 -3.467893
C    3.139519 -0.391469 -5.389310
H    4.010113 -1.045001 -5.417070
C    2.841287 0.297509 -6.504518
H    3.432431 0.186413 -7.407164
H    2.032286 1.020198 -6.547252
C    -2.038485 0.603199 -7.088937
H    -1.134499 0.081002 -7.419513
H    -1.892978 1.673053 -7.292820
H    -2.865828 0.262858 -7.720531
C    -4.895734 0.803373 -5.655848
H    -4.754831 1.480859 -6.505720
H    -5.548177 1.343017 -4.959930
C    -5.613760 -0.482208 -6.127320
H    -5.710498 -1.184709 -5.285223
H    -5.000526 -1.002420 -6.874550
C    -7.017624 -0.325605 -6.740465
O    -7.474581 -1.361054 -7.335424
O    -7.665291 0.788043 -6.587941
N    -2.910022 -3.867727 -4.456900
H    -3.116625 -2.919633 -4.738797
h    -7.082439 -3.757603 -2.228284
h    -2.647003 -4.476269 -5.205545
```

4. Additional figures

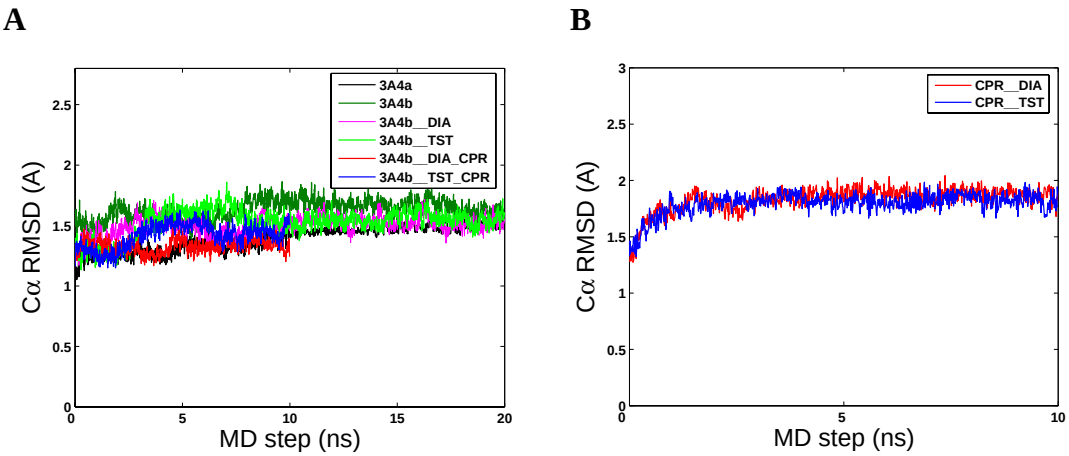


Figure S1. Cα RMSD plots of CYP3A4 in all MD simulations along time compared to their initial PDB structures. A) 3A4a, 3A4b, 3A4b_DIA, 3A4b_TST, 3A4b_DIA_CPR, 3A4b_TST_CPR are colored black, dark green, pink, green, red and blue respectively. B) Cα RMSD plots of CPR in the two CPR containing MD simulations along time compared to their initial PDB structures

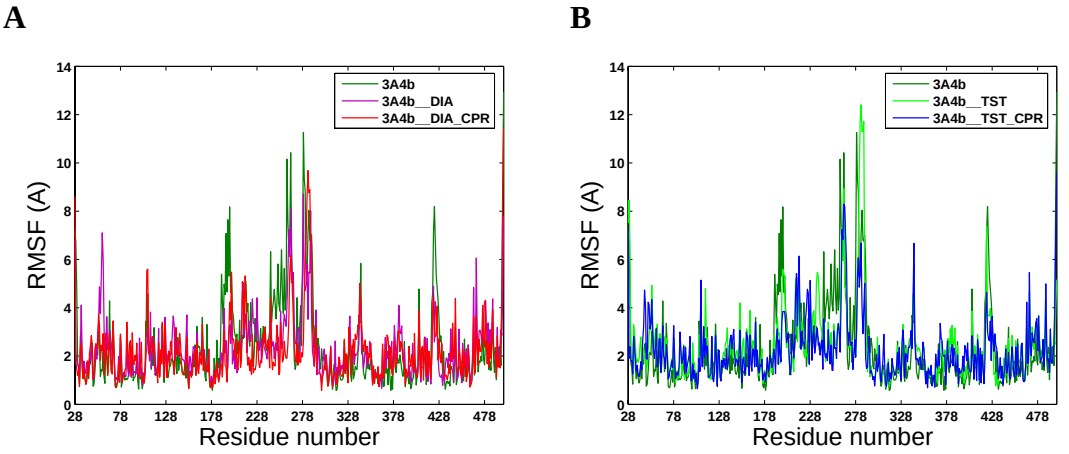


Figure S2. RMSF plots for CYP3A4 residues in all MD simulations. A) RMSF plots in unbound 3A4b (green), diazepam bound 3A4b_DIA (pink) and diazepam plus CPR bound 3A4b_DIA_CPR (red) simulations. B) RMSF plots in unbound 3A4b (dark green), testosterone bound 3A4b_TST (green) and testosterone plus CPR bound 3A4b_TST_CPR (blue) simulations.

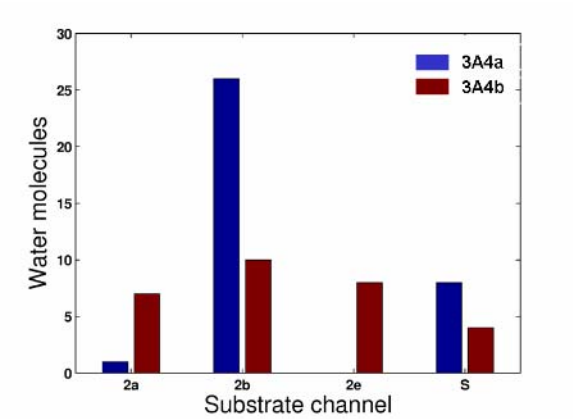


Figure S3. Number of water molecules penetrating though specific substrate access channels in the first 10ns of the unbound MD simulations. The access channels nomenclature is according ref. 4.

Explanation of the substrate channels types:

Below, the substrate access channels nomenclature follows Cojocaru et al. (Ref. 4): In the 3A4a simulation, we observed water molecules passing through channel 2b (located between the F' and G' helices, β1 sheet and the BC loop, Fig. 2 in the manuscript) and the solvent channel denoted S. This observation is supported by the CYP102A1 (CYP_{BM3}) substrate free structure in which there are between 17 and 21 water molecules along the 2b and S channels (Ref. 10). In the 3A4b simulation, we observed water molecules passing through channels 2b and S as well as through channel 2a (located between the β1 sheet and F' helix) and channel 2e that passes through the BC loop.

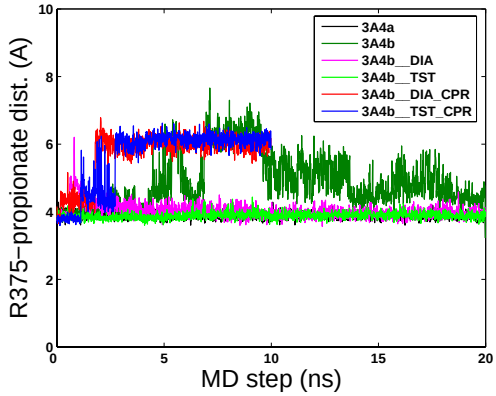


Figure S4. Distance between the heme 7-propionates and R375 all simulations. 3A4a, 3A4b, 3A4b_DIA, 3A4b_TST, 3A4b_DIA_CPR, 3A4b_TST_CPR plots are colored black, dark green, pink, green, red and blue respectively.

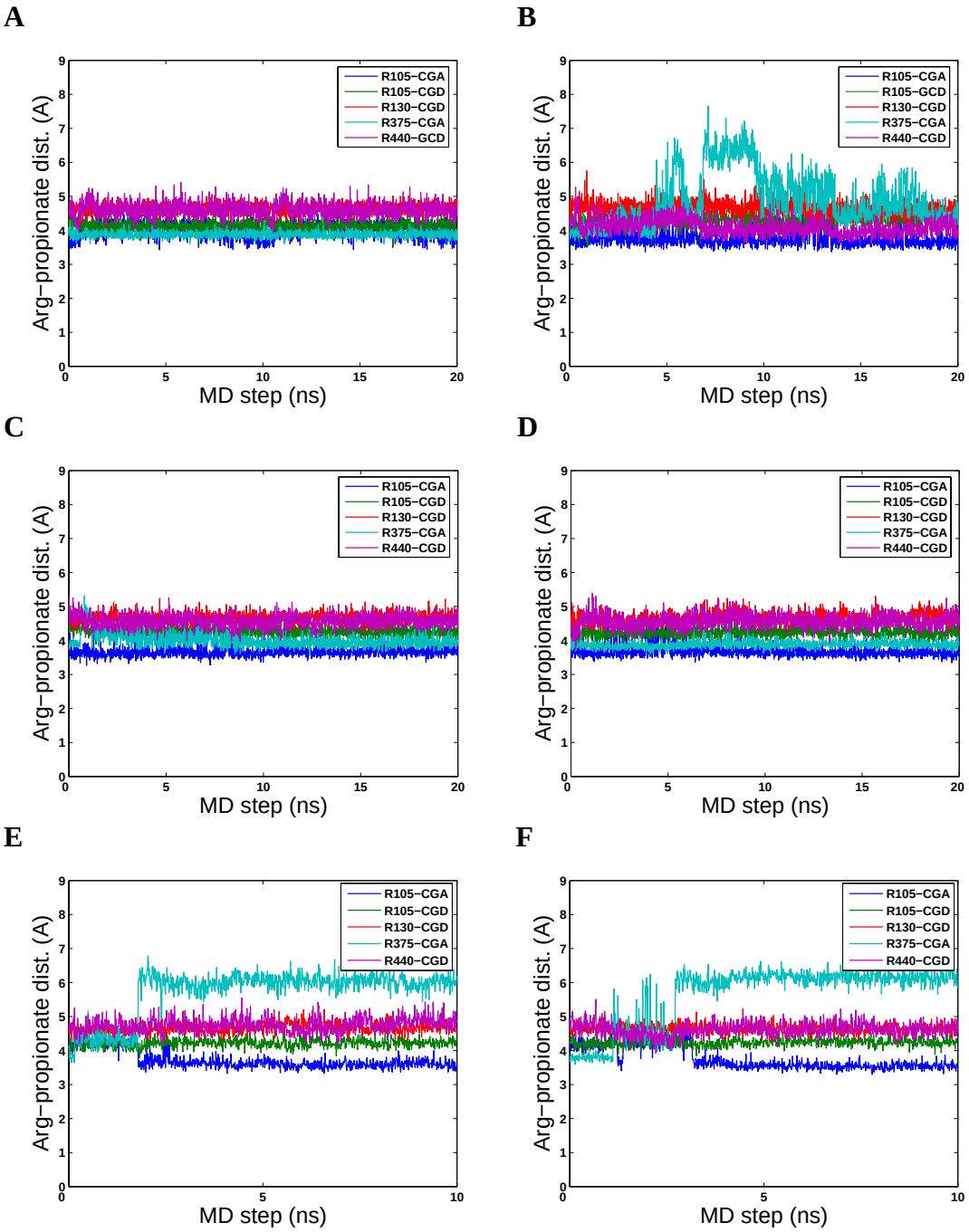


Figure S5. Distance between the heme 7-propionates and R105 (blue and green), R130 (red), R375 (cyan) and R440 (purple) along the following MD simulations: A) 3A4a; B) 3A4b; C) 3A4b_DIA; D) 3A4b_TST; E) 3A4b_DIA_CPR and F) 3A4b_TST_CPR.

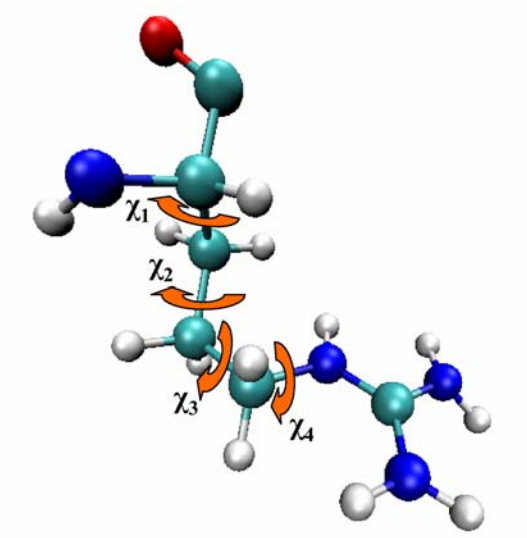


Figure S6. χ side chain torsion angles of R375.

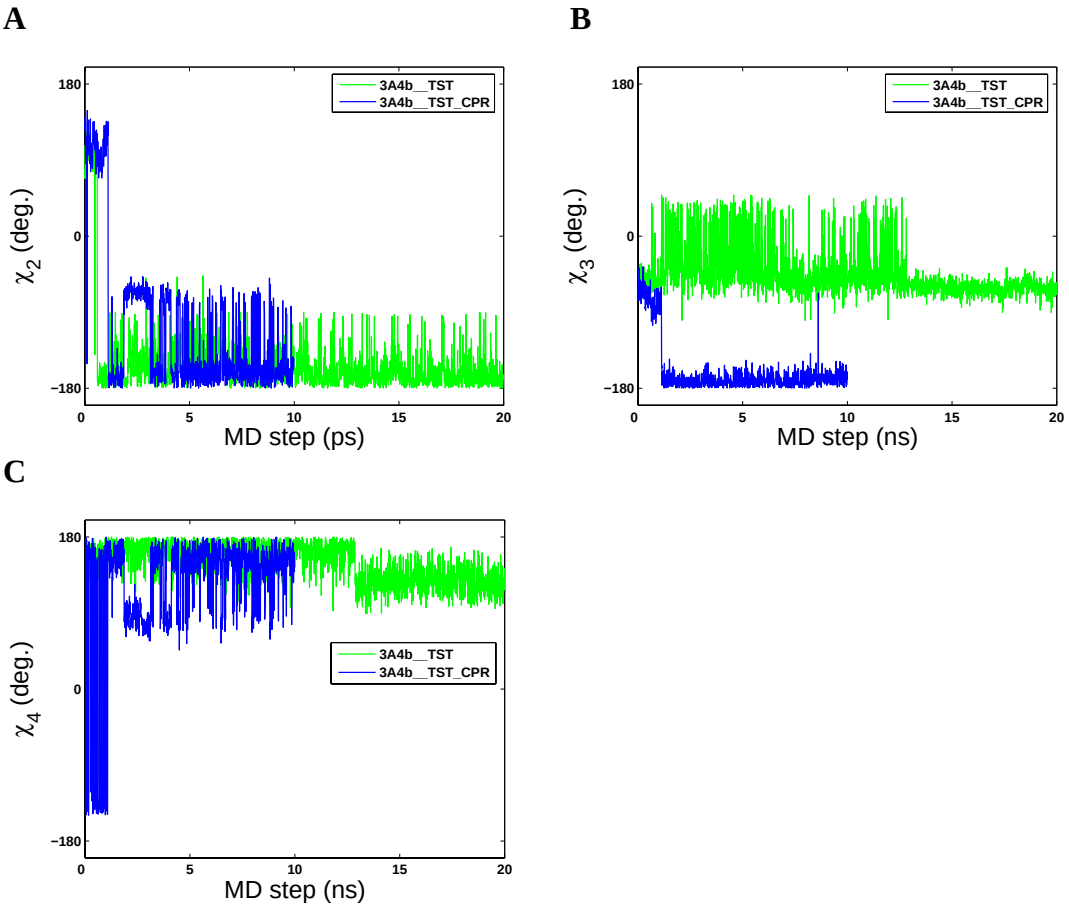


Figure S7. χ side chain torsion angles of R375 along the testosterone (green) and testosterone plus CPR (blue) containing MD simulations (A) χ_2 (B) χ_3 (C) χ_4.

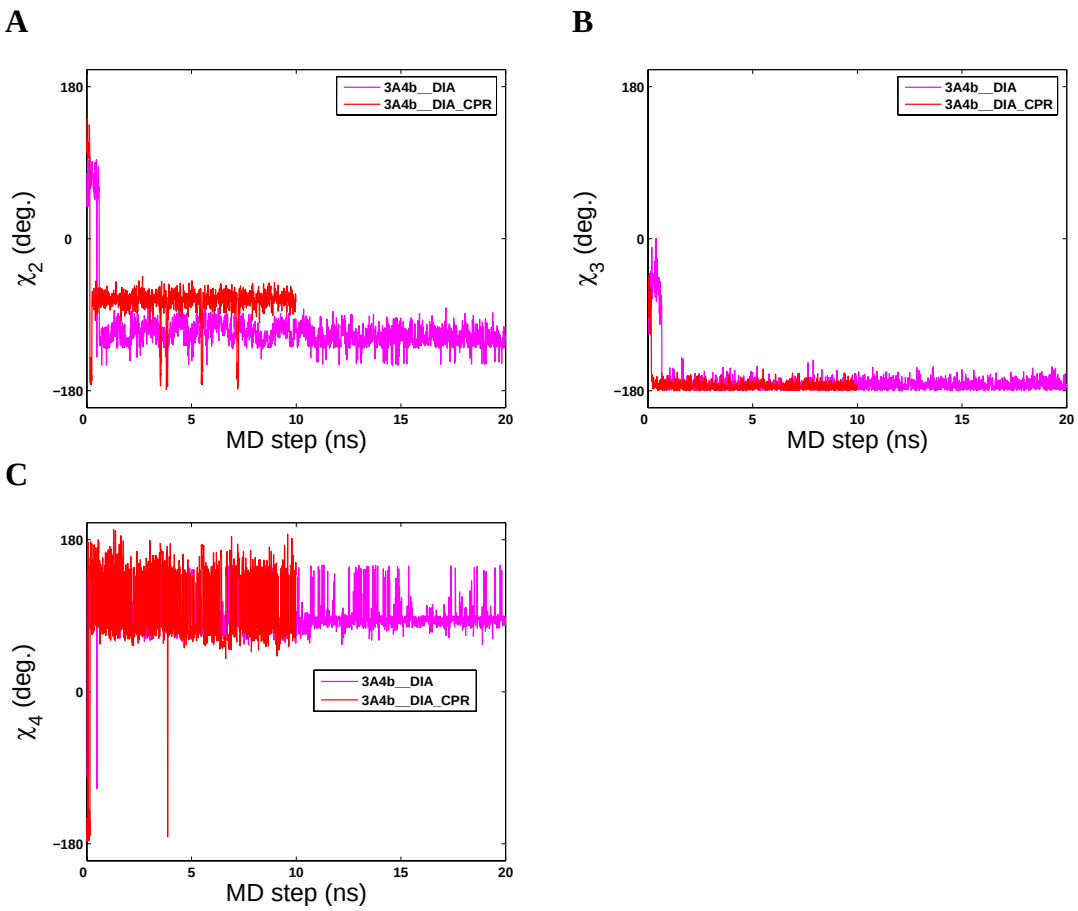


Figure S8. χ side chain torsion angles of R375 along the diazepam (pink) and diazepam plus CPR (red) containing MD simulations (A) χ_2 (B) χ_3 (C) χ_4.

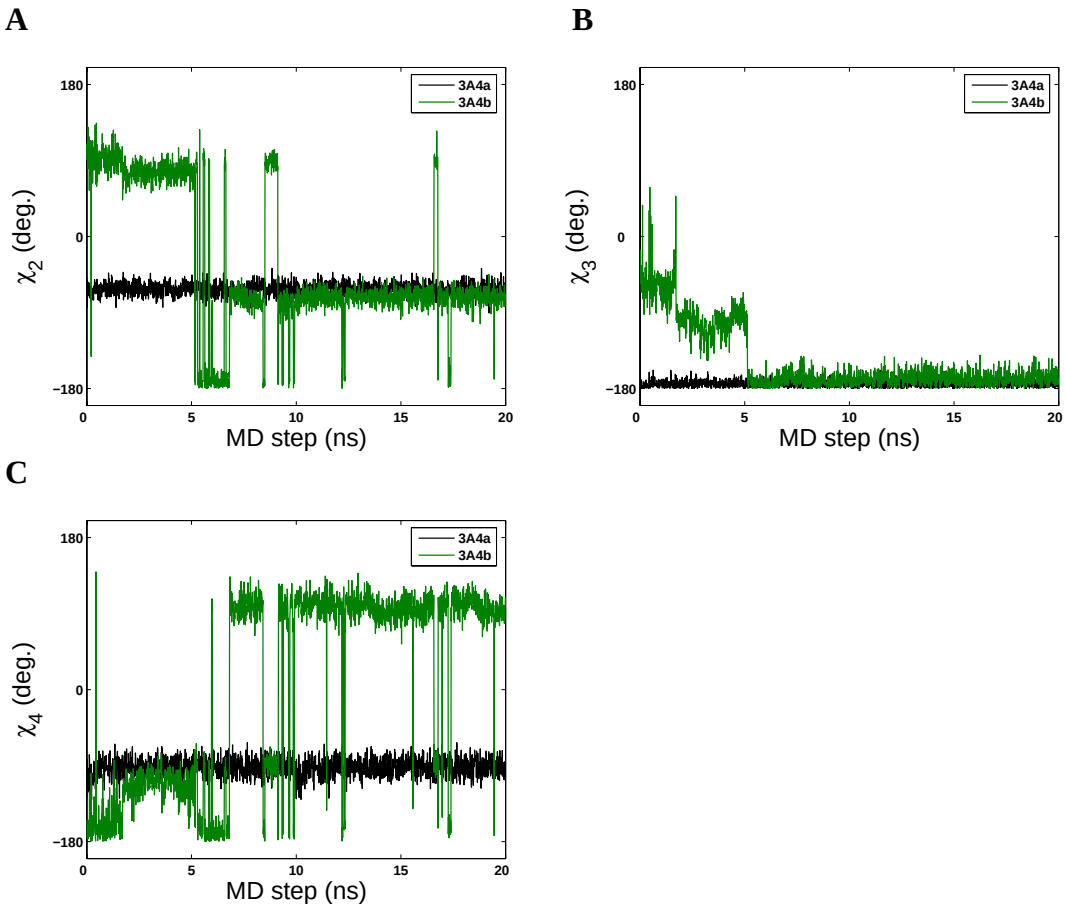


Figure S9. χ side chain torsion angles of R375 along the substrate free MD simulations. CYP3A4 conformers are colored black and green for the 3A4a and 3A4b conformers. (A) χ_2 (B) χ_3 (C) χ_4.

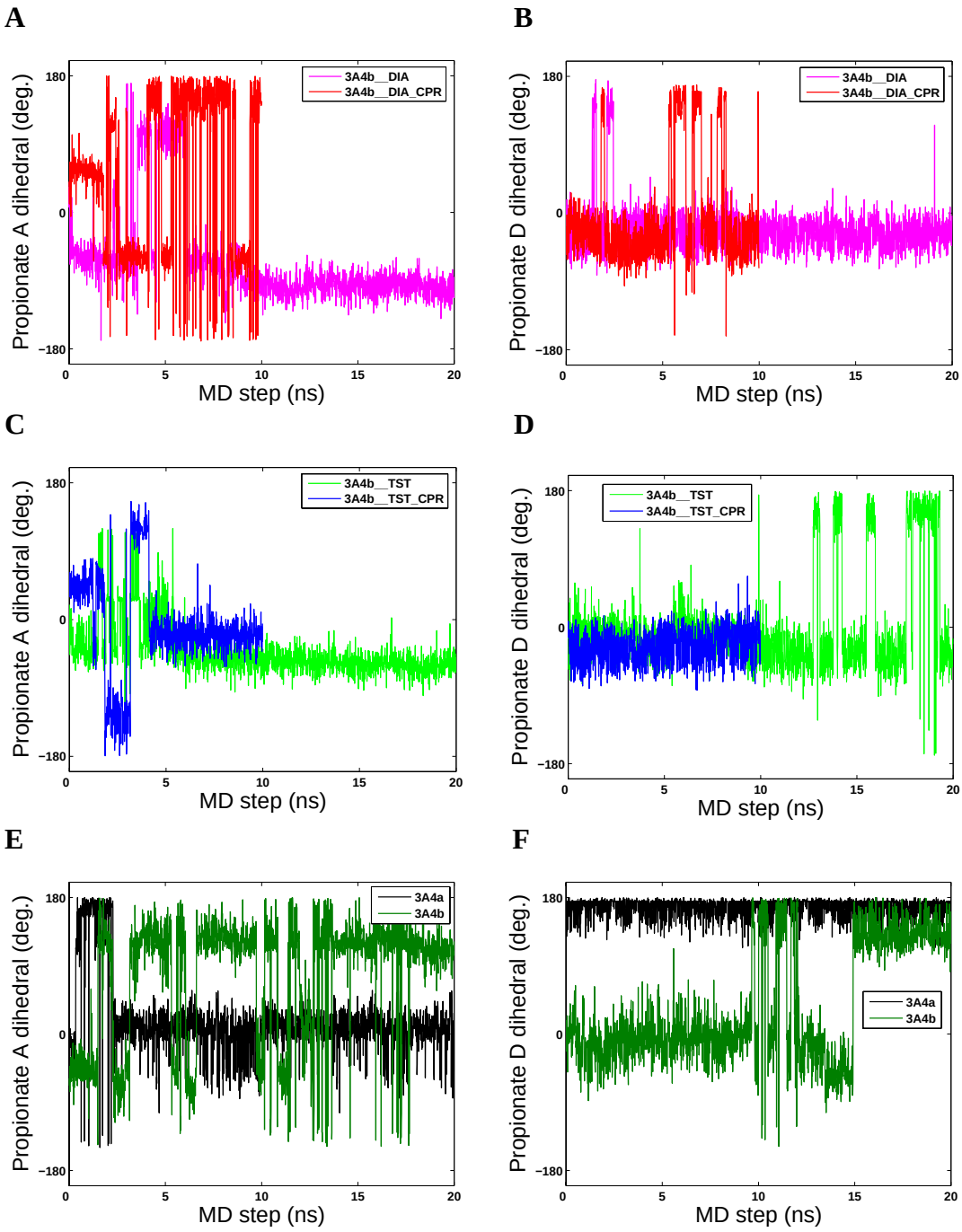


Figure S10. Propionates dihedral angles along MD simulations. A) Propionate A (7-propionate) dihedral angle along the diazepam containing MD simulations with CPR (red) and without CPR (pink). B) Propionate D (6-propionate) dihedral angle along the diazepam containing MD simulations with CPR (red) and without CPR (pink). C) Propionate A (7-propionate) dihedral angle along the testosterone containing MD simulations with CPR (blue) and without CPR (green). D) Propionate D (6-propionate) dihedral angle along the testosterone containing MD simulations with CPR (blue) and without CPR (green). E) Propionate A (7-propionate) dihedral angle along the substrate free MD simulations. F) Propionate D (6-propionate) dihedral angle along the substrate free MD simulations.

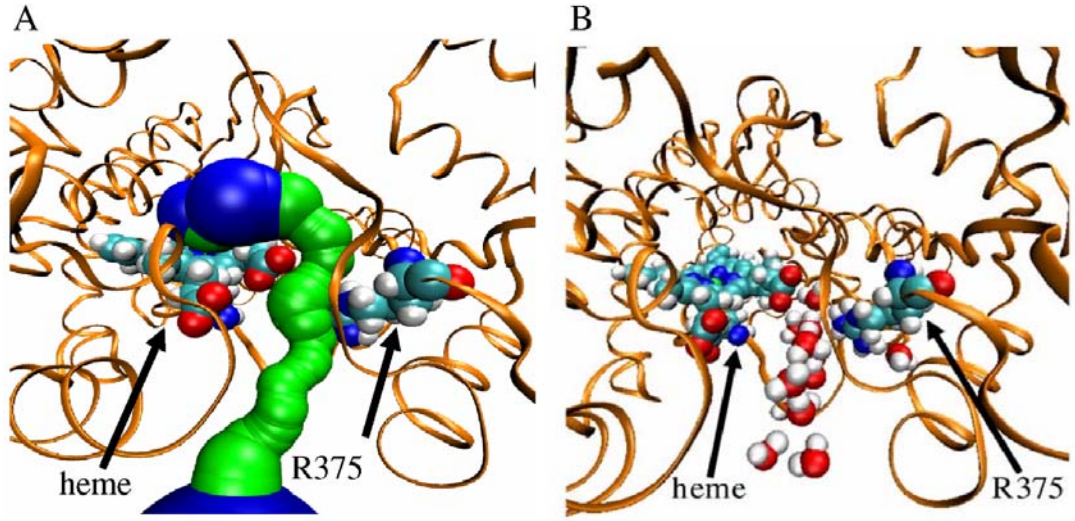


Figure S11. A) The surface of the aqueduct as calculated by MolAxis. Green spheres indicate channel radius under 2.7Å. The heme and R375 are represented as VDW spheres. The protein is colored orange. B) Explicit representation of the aqueduct with the ordered water molecules shown outside the active site. The heme, R375 and water molecules are represented by VDW spheres. Both figures share the same view.

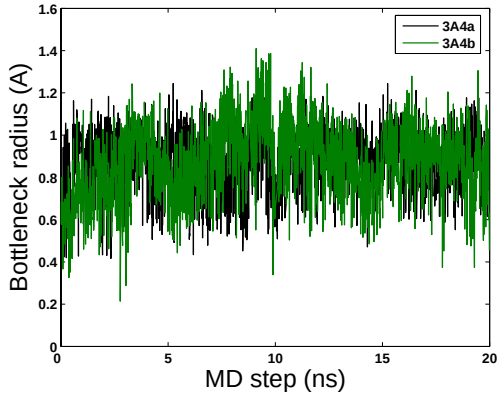


Figure S12. Aqueduct bottleneck radii along the unbound simulations 3A4a (black) and 3A4b (green).

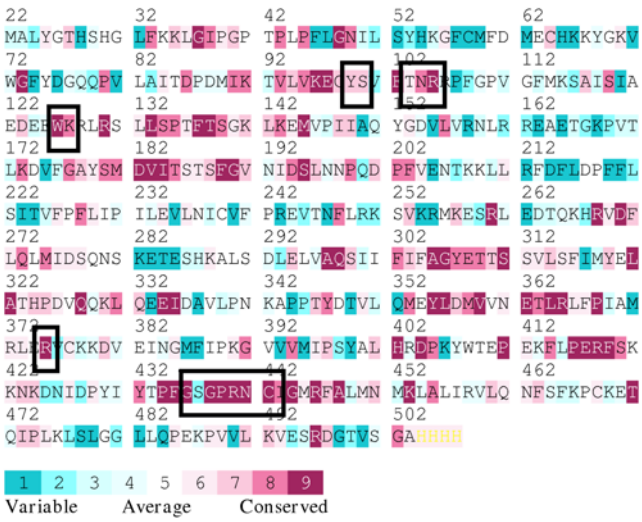


Figure S13. CYP3A4 sequence conservation calculated by ConSeq. The amino acids are colored according to the conservation scale at the bottom of the figure. Aqueduct residues are in black squares.

```
                HHHHH H                    HHHHHHHHHHHHH       S  SSS
1akd    272> QELIQRP-------------------ERIPAACEELLRRFSLVADG--RIL
                HHHHHHHHHHH      HHHH      HHHHHHHHHHHHHHH      SSSSS
1cpt    277> GGAIIGLSRNP-EQLALAKSD----PALIPRLVDEAVRWTAPVKSFMRTA
                HHHHHHHHHHHHH    HHHH      HHHHHHHHHHHHH        SSSSS
1ea1    280> AAVIDELDELYGDGRSVSFHALRQIPQLENVLKETLRLHPPLII-LMRVA
                HHHHHH                      HHHHHHHHHH       SS SS
1f4t    232> QRIREEN-------------------LYLKAIEEALRYSPPVMRTV-RK-
                HHHHH H                    HHHHHHHHHHHH       SS SSS
1jip    265> ALVRRDP------------------SALPNAVEEILRYIAPPETTT-RFA
                HHHHHHHHHH          HHHH  HHHHHHHHHHHHHH         SS
1nr6    318> ARVQEEIERVIGRHRSPCMQDRSRMPYTDAVIHEIQRFIDLLPTNLPHAV
                HHHHH                       HHHHHHHHHH       SSSSSS
1odo    266> ALVRKGE------------------VTWADVVEETLRHEPAVKHLPLRYA
                HHHHHHHHHH         HHHHHH HHHHHHHHHHHHH         SS
1r9o    321> AKVQEEIERVIGRNRSPCMQDRSHMPYTDAVVHEVQRYIDLLPTSLPHAV
                HHHHHHHHHH         HHHHHH HHHHHHHHHHHHH         SS
1suo    322> ERVQKEIEQVIGSHRPPALDDRAKMPYTDAVIHEIQRLGDLIPFGVPHTV
                HHHHHHHHHH         HHHHH    HHHHHHHHHH        SSSS
1tqn    329> QKLQEEIDAVLPNKAPPTYDTVLQMEYLDMVVNETLRLFPIAMR-LERVC
```

Figure S14. Multiple sequence alignment based on a multiple structure alignment of several P450s including: CYP_{cam} (PDB code 1akd), CYP_{terp} (PDB code 1cpt), CYP51 (PDB code 1ea1), CYP119 (PDB code 1f4t), CYP_{eryF} (PDB code 1jip), CYP2C5 (PDB code 1nr6), CYP154a1 (PDB code 1odo), CYP2C9 (PDB code 1r9o), CYP2B4 (PDB code 1suo), CY3A4 (PDB code 1tqn). Secondary structure elements are listed as follows: H- α-helix, S- β strand. The R375 position is highlighted and colored red.

A **B**

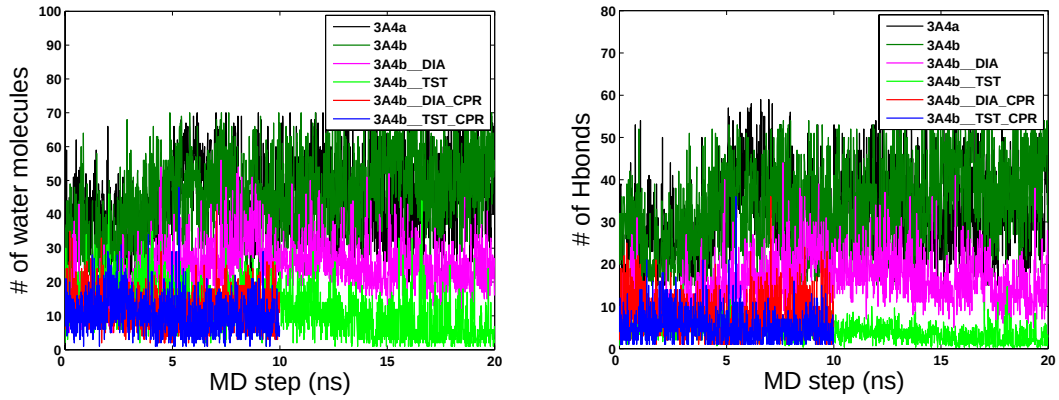


Figure S15. A) Number of active site water molecules along MD all simulations. 3A4a, 3A4b, 3A4b_DIA, 3A4b_TST, 3A4b_DIA_CPR, 3A4b_TST_CPR are colored black, dark green, pink, green, red and blue respectively. B) Number of hydrogen bonds between water molecules inside the CYP3A4 active site along all MD simulations. 3A4a, 3A4b, 3A4b_DIA, 3A4b_TST, 3A4b_DIA_CPR, 3A4b_TST_CPR are colored black, dark green, pink, green, red and blue respectively.

Explanation to how active site water molecules were detected and hydrogen bonds were calculated:

The space of the active site was defined as follows: We identified all channels emanating from the active site by MolAxis, and the active site boundaries were defined as the bottlenecks of all the channels. Then, we detected water molecules located inside all the channels spheres and these were assigned as active site water molecules. We considered a hydrogen bond between two water molecules if they meet two criteria: An O---O length shorter than 3.1 Å and an O-H---O angle greater than 146^{o}, according to Khan et al. (Ref. 41).

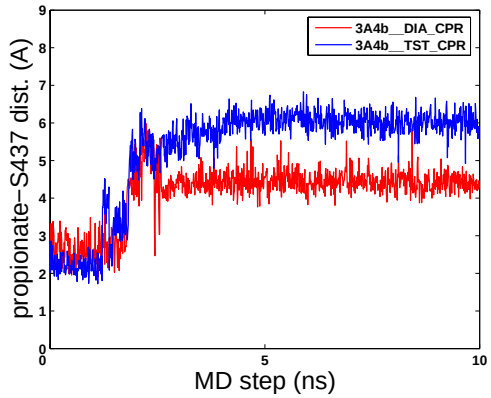


Figure S16. Hydrogen bond distance between the S437 carbonyl group and the 7-propionate in the 3A4b_DIA_CPR (red) and 3A4b_TST_CPR (blue) MDs.

Table S1. CYP3A4-CPR key interacting residues in descending order according to number of close contacts.

3A4b_DIA_CPR	3A4b_TST_CPR
LYS424_TRP574	LYS424_TRP574
PRO135_ASP499	SER131_ARG498
TYR99_THR515	ARG446_FMN631
ARG446_THR496	TYR99_THR515
ARG130_GLU494	ARG130_ARG498
LYS421_ASN573	PRO429_FMN631
SER139_TYR613	ASN441_MET490
THR138_ASP499	PRO135_ARG498
PRO439_TYR486	VAL95_ASN489
SER131_VAL513	LYS143_PHE609
ILE443_GLU494	ARG446_THR492
SER139_PHE609	GLU283_MET505
THR138_THR496	PRO135_ASP499
TYR99_LEU516	LEU351_ASN573
PRO439_ASN543	ARG130_GLU494
ASP425_TRP574	ASP425_THR576
SER134_ARG498	LYS96_ASP542
SER134_GLU494	LYS91_ASP542
SER139_THR496	MET445_FMN631
ARG446_THR492	GLY438_MET490
ILE443_GLY495	ARG446_ASP571
LYS96_ASP542	THR284_MET505
LEU351_ASP607	SER134_ARG498
ASN441_GLY491	PRO439_SER488
GLY438_ASN489	THR138_THR492
LYS424_ASN573	SER134_GLY495
ASP425_ASN537	LYS96_ASN543
PRO429_FMN631	ASN441_GLY491
SER437_ASN489	LYS424_ALA575
PRO429_ASN489	VAL95_ASN543
GLU283_MET505	LEU351_SER606
VAL95_ASP542	PRO135_GLY495
MET445_MET490	THR138_THR496
ARG446_SER606	GLY140_PHE609
SER134_ASP499	PRO439_ASN543
LYS143_GLU610	SER437_MET490
PRO429_MET490	PRO429_MET490
ASN441_MET490	GLU285_PRO511
ASP425_THR576	TYR99_TYR486
PHE435_MET490	SER134_GLU494
VAL95_ASN543	TYR430_ASN537
VAL95_ASN489	ASP428_ASN537
LYS127_THR515	LYS127_THR515
SER286_MET505	GLU285_ALA510

Table S2: positive residues that salt bridge the propionates moieties of the heme in different species.

P450	PDB code	6-propionate	7-propionate
CYP108 (terp)	1CPT	H110, R114, H375	R319
CYP101 (cam)	1AKD	R112, H355	R299
Cyp107A1 (ERYF)	1JIP	H98, R102, H349	R293
CYP102 (BM-3)	1FAG	K69, H100, R398	K69
CYP2B4	1SUO	R125, R434	R98, H369
CYP2C9	1R9O	R124, R433	R97, H368
CYP2C5	1NR6	R124, R430	R97, H365
CYP3A4	1TQN	R105, R130, R440	R105, R375

5. Calculated *PKa* values (titratable groups)

Amino acid	Charge
NTR+_0028_	1
HIS+_0028_	0
HIS+_0030_	0
LYS+_0034_	1
LYS+_0035_	1
TYR-_0053_	0
HIS+_0054_	0
LYS+_0055_	1
CYS-_0058_	0
ASP-_0061_	-1
GLU-_0063_	-1
CYS-_0064_	0
HIS+_0065_	0
LYS+_0066_	1
LYS+_0067_	1
TYR-_0068_	0
LYS+_0070_	1
TYR-_0075_	0
ASP-_0076_	-1
ASP-_0086_	-1
ASP-_0088_	-1
LYS+_0091_	1
LYS+_0096_	1
GLU-_0097_	-1
CYS-_0098_	0
TYR-_0099_	0
ARG+_0105_	1
ARG+_0106_	1
LYS+_0115_	1
GLU-_0122_	-1
ASP-_0123_	-1
GLU-_0124_	-1
GLU-_0125_	-1
LYS+_0127_	1
ARG+_0128_	1
ARG+_0130_	1
LYS+_0141_	1
LYS+_0143_	1
GLU-_0144_	-1
TYR-_0152_	0
ASP-_0154_	-1
ARG+_0158_	1
ARG+_0161_	1
ARG+_0162_	1
GLU-_0163_	-1
GLU-_0165_	-1
LYS+_0168_	1

LYS+_0173_	1
ASP-_0174_	-1
TYR-_0179_	0
ASP-_0182_	-1
ASP-_0194_	-1
ASP-_0201_	-1
GLU-_0205_	-1
LYS+_0208_	1
LYS+_0209_	1
ARG+_0212_	1
ASP-_0214_	-1
ASP-_0217_	-1
GLU-_0234_	-1
CYS-_0239_	0
ARG+_0243_	1
GLU-_0244_	-1
ARG+_0250_	1
LYS+_0251_	1
LYS+_0254_	1
ARG+_0255_	1
LYS+_0257_	1
GLU-_0258_	-1
ARG+_0260_	1
GLU-_0262_	-1
ASP-_0263_	-1
LYS+_0266_	1
HIS+_0267_	0
ARG+_0268_	1
ASP-_0270_	-1
ASP-_0277_	-1
LYS+_0282_	1
GLU-_0283_	-1
GLU-_0285_	-1
HIS+_0287_	0
LYS+_0288_	1
ASP-_0292_	-1
GLU-_0294_	-1
TYR-_0307_	0
GLU-_0308_	-1
TYR-_0319_	0
GLU-_0320_	-1
HIS+_0324_	0
ASP-_0326_	-1
LYS+_0330_	1
GLU-_0333_	-1
GLU-_0334_	-1
ASP-_0336_	-1
LYS+_0342_	1
TYR-_0347_	0
ASP-_0348_	-1
GLU-_0354_	-1

TYR-_0355_	0
ASP-_0357_	-1
GLU-_0362_	-1
ARG+_0365_	1
ARG+_0372_	1
GLU-_0374_	-1
ARG+_0375_	1
CYS-_0377_	0
LYS+_0378_	1
LYS+_0379_	1
ASP-_0380_	-1
GLU-_0382_	-1
LYS+_0390_	1
TYR-_0399_	0
HIS+_0402_	0
ARG+_0403_	1
ASP-_0404_	-1
LYS+_0406_	1
TYR-_0407_	0
GLU-_0410_	-1
GLU-_0412_	-1
LYS+_0413_	1
GLU-_0417_	-1
ARG+_0418_	1
LYS+_0421_	1
LYS+_0422_	1
LYS+_0424_	1
ASP-_0425_	-1
ASP-_0428_	-1
TYR-_0430_	0
TYR-_0432_	0
ARG+_0440_	1
CYS-_0442_	0
ARG+_0446_	1
LYS+_0453_	1
ARG+_0458_	1
LYS+_0466_	1
CYS-_0468_	0
LYS+_0469_	1
GLU-_0470_	-1
LYS+_0476_	1
GLU-_0486_	-1
LYS+_0487_	1
LYS+_0492_	1
GLU-_0494_	-1
ARG+_0496_	1
ASP-_0497_	-1
CTR-_0499_	-1
PAA-_0500_	-1
PDD-_0500_	-1

We used DelPhi (Rocchia W, Alexov E, Honig B (2001) Extending the applicability of the non-linear Poisson–Boltzmann equation: multiple dielectric constants and multivalent ions. *J Phys Chem B* 105:6507-6514) as a Poisson-Boltzmann solver with a dielectric constant of 4